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Cervicovaginal ureaplasmal infection alone is not predictive of preterm birth. Only a subpopulation of women infected in the lower genital tract are at risk for chorioamnion invasion and premature birth. The major goal of this study was to identify microbiologic factors that may predispose to and/or predict chorioamnion invasion and premature birth. Specifically, we sought to determine if the presence of bacterial vaginosis (BV) was a risk factor for ureaplasmal invasion of the chorioamnion. At the first prenatal visit, cervicovaginal ureaplasmal colonization nor BV alone were predictors of chorioamnion invasion but women colonized with both ureaplasma and BV were 2.8 times more likely to have an intrauterine fetal demise. We also sought to determine if ureaplasma chorioamnion colonization was associated with premature birth in active duty military personnel and whether this explains the observed difference in the rate of prematurity between active duty and dependent women. After controlling for all variables in the multivariate analysis, enlisted women were 2.4 times more likely to have a preterm birth < 34 weeks than women that were not enlisted. However, chorioamnion infection did not explain the differences. Results from this study have given us a comprehensive analysis of the incidence of sexually transmitted pathogens in pregnant women in the Navy. Future work will include thet speciation of ureaplasma isolates to determine if virulence of one speciesis predictive of chorioamnion invasion.

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INTRODUCTION

Women are playing increasing roles in active duty military service and suffering unexplained adverse outcomes of pregnancy. In the Navy, up to 20% of enlisted women become pregnant each year and nearly 15% of these pregnancies suffer adverse effects. This percentage is in excess of that experienced in most other US female populations and despite a number of preliminary investigations, the difference does not appear to be related to environmental exposures.

Preterm birth complicates 8-10% of all pregnancies in the U. S. and is the leading cause of infant morbidity and mortality in the United States. Some of the traditional factors associated with adverse outcomes of pregnancy such as limited access to prenatal care or poor nutritional status are not operative among naval personnel. Nonetheless, at least 10% of Navy live births are premature (< 37 weeks gestation) or low birth weight (<2,500 grams). In addition, previous studies have indicated among pregnant, enlisted women, spontaneous abortions occur in 9.9%, 2.7% of pregnancies are ectopic and 1.5% result in fetal death (0.7% early and 0.8% late). Bacterial infections of the lower genital tract may in part explain poor pregnancy outcome. Investigators have also shown that bacterial vaginosis in the presence of *Mycoplasma hominis* is associated with preterm delivery of a low-birth weight infant.

We have previously shown that *Ureaplasma* species is the single most common microorganism isolated from the chorioamnion of women in spontaneous labor with intact membranes and in whom there are no chances for cervicovaginal contamination of the placenta (i.e. they delivered by cesarean section with intact membranes). Furthermore, ureaplasmal infection of the chorioamnion in the absence of other bacteria was associated with birth < 37 weeks and birth $\leq$ 34 weeks in women with spontaneous onset of labor even after multifactorial analysis to adjust for labor and other obstetric and demographic factors that could confound the association. Infection was inversely related to gestational age and birth weight. Other related studies indicate that ureaplasmal infection is a significant cause of respiratory disease, meningitis and death in very low birth weight infants.

The major goal of the present study was to determine if the increased adverse pregnancy outcomes in active-duty versus non-active duty females was due to differences in BV or ureaplasmal or other microbial amnion infection of the chorioamnion. A secondary objective was to determine if BV predisposes to ureaplasma infection of the chorioamnion.

BODY

METHODS

Study population

All pregnant women seeking medical care at the prenatal clinic of the Naval Medical Center San Diego were invited to participate in the study. These women were all dependents of active duty military personnel or active duty military personnel and thus eligible for health care.

Approximately 2% (n=106) of the women who participated in our study became pregnant more than once during the 3-year enrollment period, and granted informed consent each time. These women were followed for each pregnancy and considered as independent pregnancy events.

Objectives and Outcomes

The study's primary objective was to determine whether chorioamnion infection, in particular infection by *Ureaplasma* species, was associated with premature birth in active duty women and non-active duty women and could potentially explain the increased percentage of adverse pregnancy outcomes observed by others in active duty military personnel. A secondary objective was to identify risk factors for chorioamnion invasion and premature birth. Specifically, we sought to determine if bacterial vaginosis (BV) was a risk factor for ureaplasma invasion of the chorioamnion.

The following adverse pregnancy outcomes were studied: spontaneous abortion (SAB), premature birth (defined as either <37 weeks gestation, or ≤34 weeks gestation, based upon sonography when available or on dates of last menstrual period), stillbirth (IUFD), and low birth weight (defined as either <2,500 grams, or ≤1,000 grams).

Data Collection

Upon the first visit for prenatal care at Naval Medical Center, San Diego (NMCSD), both active-duty and non-active duty pregnant women were invited to participate in this study. After the study was explained and informed consent was granted, study volunteers were asked to complete an enrollment questionnaire, and to permit a vaginal swab and collection of a 10cc blood specimen in conjunction with other routine clinical specimen collections.

Study subjects' health records were marked such that when they were about to deliver, research staff were notified and a number of clinical specimens were collected: vaginal swabs for Gram stain for BV, vaginal culture for mycoplasmas and sera. Whenever possible, nasal swabs from infants were also obtained. Occasionally, cerebral spinal fluid

and respiratory secretions were collected and cultured if the babies attending physicians requested.

We attempted to collect and study all placentas from mothers who delivered by C-section, and those who had a preterm delivery. In addition, women delivering vaginally were randomly selected for comparison. The placentas were processed within one hour of delivery and were evaluated by aerobic and anaerobic culture and culture for ureaplasmas and mycoplasmas.

Questionnaires

Six questionnaires were developed to collect demographic, laboratory, health history, and delivery outcome data. To reduce data entry errors all questionnaires were color coded, prepared in a mark-sense format (for scanning) and linked by a unique study identification number sequentially assigned to each study subject (Table 1).

Table 1

Questionnaire Name	Color	Completed by:	Summary of Contents
Enrollment	Blue	All study participants	Captured self-reported demographic and health-history data on the mother
1st Prenatal Visit Laboratory	Burgundy	Lab personnel for all study participants	Recorded prenatal specimen collection results
Outcome Questionnaire	Orange	Nurse coordinator for all study participants	Captured outcome of the pregnancy, and other birth-related information taken from the delivery lot (when available)
Mother's Data at Delivery	Purple	Nurse coordinator for those in the placenta group	Captured data from the hospital's medical record relating to the woman's pregnancy history, prenatal illnesses, and type of delivery
Infant's Data at Delivery	Green	Nurse coordinator for those in the placenta group	Captured data from the hospital's medical record relating to the infant's demographics, birth weight, gestational age, and clinical parameters of the birth process
Laboratory Data at Delivery	Brown	Lab personnel for those in the placenta group	Recorded vaginal swab results, chorioamnion swab results, placenta tissue results

Laboratory procedures

Clinical specimens collected at the time of enrollment for all consenting women included 6 vaginal swabs for BV assessment, mycoplasma and ureaplasma cultures, *Trichomonas vaginalis* culture, and mucosal antibodies. Serum was also obtained. Vaginal secretions for BV and maternal blood were collected prior to delivery from women in one of the study groups (all women delivering by cesarean section, all preterm deliveries, and randomly selected vaginal deliveries). Placental tissues and cord blood were collected asceptically at the time of delivery and placed in sterile plastic bags or syringes. Nasal secretions from each infant born to a study mother were also collected and studied for ureaplasma and mycoplasma colonization. Within one hour of collection, specimens were taken to the laboratory and were cultured for aerobes and anaerobes. Specimens were inoculated into mycoplasma and ureaplasma transport media and were frozen at –70°C until shipment to the Diagnostic Mycoplasma Laboratory at the University of Alabama at Birmingham.

Great care was taken to reduce the chance of contamination in the culturing of placental tissues. Processing took place in a biosafety cabinet. Placentas were placed on a sterile surface and using sterile gloves examined for anatomical malformations or lesions suggestive of infection. Using aseptic technique, incisions were made on the fetal surface of the placenta and the amnion was separated from the chorion using sterile surgical instruments. Membrane specimens were obtained by swabbing the interface between the chorion and the amnion. Three sites from the chorioamnion were sampled with priority given to areas where abnormalities were present. The chorioamnion swabs were inoculated into 3.0 ml of sucrose phosphate buffer with 10% fetal calf serum (2SP). Half of the inoculum (1.5ml) was cultured on aerobic media and mycoplasmal and ureaplasmal transport media while the remaining 1.5ml was transported to the anaerobic chamber for further processing.

The placental tissue (at least 3 sites sampled) was collected with sterile forceps and scissors and placed into a sterile petri dish containing 3.0 ml of 2SP for aerobic cultures. Half of the placental tissue sampled was placed into pre-reduced medium for transport to an anaerobic chamber. Further, the aerobic processing of the placental tissue included the mincing of tissues with sterile scissors and inoculation of this tissue suspension into the appropriate media for culture of aerobic bacteria including transport media for ureaplasma and mycoplasma. The media used for aerobic culture of the chorioamnion interface and placental tissue included Becton Dickinson's Trypticase Soy Agar with 10% Sheep Blood, V Agar, and Chocolate II Agar with Hemoglobin & IsoVitaleX. Also, a Chocolate II Agar with Hemoglobin & IsoVitaleX was inoculated from the chorioamnion inoculum and placental tissue suspension to be incubated in a reduced oxygen atmosphere (10% CO_2, 5% O_2, 85% N_2). Anaerobic cultures were processed in an anaerobic chamber, Bactron II. The placental tissue from the anaerobic transport media was aseptically processed by mincing with sterile instruments in 3 ml of 2SP. The 2SP from the chorioamnion inoculum and the placental tissue suspension were plated to the appropriate medias for culture of anaerobic bacteria. The anaerobic media onto which the specimens were plated was Pre-Reduced Anaerobically Sterilized Bacteroides

Bile Esculin Agar, Brucella Blood Agar with Vitamin K & Hemin, Brucella Laked Blood Kanamycin Vancomycin, Phenylethyl Alcohol Blood Agar, and inoculated Thioglycolate Medium with Hemin, Vitamin K & Calcium Carbonate from Anaerobe Systems.

Aerobic cultures were incubated at 36.5°C in 7% CO_2, and checked daily for five days. Anaerobic cultures were incubated at 36.5°C for seven days. The reduced oxygen culture was incubated at 36.5°C. and checked on day three and day seven. At day seven, no growth reduced oxygen cultures and anaerobic cultures were discarded. Negative cultures were blind passed aerobically to Chocolate II and anaerobically from the Thioglycolate Medium on the fifth day to detect any microorganisms which failed to grow on the original plates. All bacteria were isolated and identified using standard biochemical or enzymatic tests.

Mycoplasma cultures were frozen at –70°C and were shipped on dry ice to the Diagnostic Mycoplasma Laboratory monthly. Once the specimens were thawed, serial dilutions were made in 10 B and SP4 broth. 20 µl aliquots of the original specimen and of each aliquot were plated on the appropriate agar (A8 for 10 B and SP4 for SP4). Organisms routinely sought were *Ureaplasma* species, *M. hominis*, and *M. genitalium*. Dilutions of broth were incubated at 37°C in room air (14 days for 10 B and 6 weeks for SP4). Broths were read daily for detection of a color change. Plates were incubated at 37°C in 5% CO_2 (14 days for A8 and 6 weeks for SP4). Plates were read three times each week prior to being reported out as negative. Negative SP4 broth cultures were blind passed (broth to agar inoculation) between days 10 to 21 to increase the chance of isolating *M. genitalium*.

Supplemental data

In an effort to assess the representativeness of our study sample, we sought to compare the demographics of our enrolled population with that of the total population of women who sought prenatal care at NMCSD during the study enrollment period. Demographics studied included: age, race, and active duty status.

In an effort to assess the subject's routine chlamydial and gonococcal culture results and prenatal antibiotic use, the social security numbers of study participants were used to extract clinical laboratory results and pharmacy data from the hospital's Composite Health Care System database. Supplemental data were selected for extraction based on the subject's enrollment date and estimated date of confinement.

Statistical analyses

Univariate and multivariate modeling of outcome data were performed with standard techniques. Covariates included: maternal race/ethnicity, age, marital status, education level, smoking history, prenatal antibiotic use, presence or absence of BV at prenatal and at delivery, mycoplasma, ureaplasma, *T. vaginalis*, service member's pay grade and delivery type. Chi squared tests were used to determine eligible covariates for multivariate modeling. Covariates with a p-value less than 0.15 were included. Pair-wise comparisons were performed to assess collinearity. Backward elimination procedure was

used in multivariate analyses. Data management and all statistical analyses were performed using the SAS© system software (Version 6.12, Cary, North Carolina).

KEY RESEARCH ACCOMPLISHMENTS

From June 1996 to April 1999, 4,313 pregnant active duty and dependent women from among an estimated 13,588 potential subjects granted informed consent and were enrolled in the study. Many women were not offered study enrollment as they were first seen in other clinics or they were transferred to other prenatal clinics before attending their scheduled appointment. Thus, the potential subject figure is falsely inflated by an unknown amount.

The majority of study participants were: of white race (54%), married (84%), at least a high school education (41%), a military dependent (75%), between 20-24 years of age (37%), and at least 7 weeks pregnant at the time of enrollment (52%). Study subjects were very similar to all pregnant women seeking prenatal care during the study enrollment period (Table 2). Slightly more regular active-duty mothers participated in the study than expected.

Table 2

A comparison of the study subjects vs. the total population of women who sought prenatal care at the Naval Medical Center San Diego during study enrollment period.

Covariate	UAB Sample (N=4,291)* N (%)	Target Population (N=13,588) N (%)	*p*-value**
Maternal age(years)***			
≤ 19	512 (11.97)	1676 (12.33)	.48
20-24	1574 (36.80)	4913 (36.16)	.53
25-29	1164 (27.22)	3617 (26.62)	.51
30-34	681 (15.92)	2218 (16.32)	.48
≥ 35	346 (8.09)	1164 (8.57)	.30
Maternal race***			
White	2322 (54.43)	5777 (51.79)	<.0001
Hispanic	575 (13.48)	1494 (13.39)	<.0001
Asian/Pacific Islander	576 (13.50)	1936 (17.36)	.18
Black	559 (13.10)	1514 (13.57)	.001
Other	234 (5.49)	434 (3.89)	<.0001
Active Duty Status***			
Active Duty	853 (21.87)	1974 (14.79)	<.0001
Non-Active Duty	3047 (78.13)	11377 (85.21)	<.0001

*Demographic data available for only n=4,291 of the total study sample due to 22 subjects either not completing, or not returning their enrollment questionnaire.
**Chi-square, Mantel-Haenszel
***Variable totals do not always add up to the column total due to missing data. Percentages are based on non-missing data.

A total of 762 placentas were intensively studied. Mothers whose placentas were studied were fairly representative of the entire 4,291 mothers studied (Table 3). However, in general the placenta group was older and had more active duty mothers.

Table 3

A comparison of the women with placenta specimens vs. total subjects enrolled at prenatal visit.

Covariate	**Placenta Sample (N=761) N (%)**	**Study Sample (N=4,291)* N (%)**	***p*-value****
Maternal age (years)***			
≤ 19	63 (8.29)	512 (11.97)	.003
20-24	239 (31.45)	1574 (36.80)	.005
25-29	225 (29.61)	1164 (27.22)	.17
30-34	137 (18.03)	681 (15.92)	.15
≥ 35	96 (12.63)	346 (8.09)	<.0001
Maternal race***			
White	407 (53.69)	2322 (54.43)	.72
Hispanic	111 (14.64)	575 (13.48)	.39
Asian/Pacific Islander	106 (13.98)	576 (13.50)	.72
Black	100 (13.19)	559 (13.10)	.94
Other	34 (4.49)	234 (5.49)	.26
Active Duty Status***			
Active Duty	170 (25.04)	853 (21.87)	.12
Non-Active Duty	509 (74.96)	3047 (78.13)	.02

*Demographic data available for only n=4,291 of the total study sample due to 22 subjects either not filling out, or not returning, their enrollment questionnaire.
**Chi-square, Mantel-Haenszel
***Variable totals do not always add up to the column total due to missing data. Percentages are based on non-missing data.

When we examined 3 delivery outcome groups (c-section membranes intact, c-section membranes ruptured, vaginal) to assess the rates of preterm delivery by group, the study population consisted of 3,266 subjects. This total resulted after removing 22 subjects who had twins, 636 subjects whose pregnancy outcomes could not be determined, and the 46 subjects whose medical records indicated they had a spontaneous or a therapeutic abortion.

Among these 3,266 subjects, 2,732 (75.6%) delivered vaginally (8% of these were preterm based on <37 weeks and 2.4% based on $\leq$ 34 weeks), 315 (9.6%) delivering by C-section with ruptured membranes (10% of these were preterm < 37 weeks and 5.7% based on $\leq$ 34 weeks), and 219 (6.7%) delivered by C-section with intact membranes and 16% of these were preterm < 37 weeks and 8.7 % based on $\leq$ 34 weeks. There were also 0.65% of pregnancies ending in an intrauterine fetal demise (IUFD). (Figures 1 and 2 in Appendix 1).

Among the 762 women whose placentas were studied, 328 (43%) had vaginal deliveries (18.6% preterm < 37 weeks and 8.5% $\leq$ 34 weeks), 244 (32%) had C-sections with ruptured membranes (8.6% preterm < 37 weeks and 4.5 % $\leq$ 34 weeks), and 190 (25%) delivered by C-section with intact membranes (13% preterm < 37 weeks and 6.8% $\leq$ 34 weeks) (Figures 3 and 4 in Appendix I).

Univariate and Multivariate Analyses

Because of the potential confounding with respect to multiple births, 22 subjects who delivered twins were excluded from risk factor modeling analyses. Thus, the population used for the univariate and multivariate analyses was n=4,291 subjects.

Univariate analyses, including unadjusted relative risks, were completed for each of the six adverse pregnancy outcomes (Table 1 in Appendix II). Multivariate modeling indicated the strongest consistent association between prenatal antibiotic use and adverse outcomes (Table 2 in Appendix II). In 4 of the 6 models, this covariate was significant. For instance, women who used antibiotics during their pregnancy had 1.7 times (95%CI = 1.3, 2.3) the odds of a preterm pregnancy of less than 37 weeks than women who did not use antibiotics. Antibiotic users had twice (95%CI = 1.2, 3.2) the odds of a preterm pregnancy of less than or equal to 34 weeks than non-users. Antibiotic use was also found significantly associated with stillbirth and low birth weight (<2500 grams). More information is needed to determine the true significance of this data, i.e which antibiotics were given, at what point in time during the pregnancy they were given and for what reason. Some of this information may not be accessible. Blacks, Hispanics, Asian/Pacific Islanders and women of other race/ethnicities were all at increased odds of stillbirth when compared with whites. Blacks, Asian/Pacific Islanders and women of other race/ethnicities were also at increased odds of low birth weight (<2500 grams) when compared with whites. However, Hispanics were not significantly different from whites. Women 35 and older were found to have 2.5 times (CI = 1.8, 3.4) the odds of a spontaneous abortion than women younger than them. Results in the multivariate analyses are significant after adjusting for all other covariates in the model.

In looking at just the placental population, it was found that enlisted women were 2.4 times (CI = 1.3, 4.5) the odds of having a preterm delivery $\leq$ 34 weeks. This holds true after controlling for possible confounding variables. It is unclear as to what factors contributed to this association. Other than the afore mentioned association, there were little or no significance in the multivariate analysis, but it is impossible to tell whether this was real or due to small numbers (Tables 3,4,5,6 Appendix II).

Microbiological Results

Vaginal secretions taken at the first prenatal visit were assessed for the presence of *Ureaplasma* species and BV. BV smears were analyzed using the Nugent Gram stain method. Our BV rate of positivity (a score of > 7) was 21% at prenatal assessment and 17% at delivery. Trichomonas was isolated from 36/4123 patients (0.9%). These are consistent with previous reports. Ureaplasma was isolated from 2,497/4,193 (60%) and *Mycoplasma hominis* was isolated from 437/4,193 (10%), *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were diagnosed in <1% and 2.45% respectively.

One or more microorganisms was isolated from the chorioamnion of 38.6% of all deliveries (294/761) of all singleton births but in only 23/190 (12.1%) of those delivering by c-section with membranes intact. The frequency of each individual microbial species is found in Appendix III. The majority of microorganisms isolated are ones which have previously been implicated in prematurity, including *Ureaplasma* species, organisms associated with BV (*including M. hominis, G. vaginalis, Bacteroides sp.*), *Group B Streptococcus*, *Listeria monocytogenes* and *Chlamydia trachomatis*.

Ureaplasma species and *G. vaginalis* were the most common organisms isolated from the chorioamnion in the vaginal delivery group as well as in the c-section ruptured membrane group. In the c-section intact membrane group, the number of organisms isolated were too small for comparison. Onset of labor is either spontaneous with the rupture of membranes or indicated (the physician's decision to bring about delivery for maternal or fetal implications). When each of the delivery groups were further broken down into spontaneous and indicated, the number of organisms was higher in the spontaneous group. A summary of placental infection with regard to spontaneous and indicated labor is shown in Table 4.

Table 4

Placental Infection with Regard to Spontaneous Vs. Indicated Labor

	All Deliveries N=761	Vaginal Deliveries N=328	C-section Ruptured N=243	C-section Intact N=190
Ureaplasma only	p=0.0002	NSD*	p=0401	NSD
Bacteria only	p≤0.0001	NSD	NSD	NSD
BV only	NSD	NSD	NSD	NSD
Ureaplasma + Bacteria	P=0.0002	NSD	NSD	**
Ureaplasma + BV	P=0.0031	NSD	NSD	**
BV + Bacteria	P=0.0021	NSD	NSD	**
Ureaplasma + Bacteria + BV	NSD	NSD	NSD	**
Any Ureaplasma	p≤0.0001	NSD	p=0.0362	NSD
Any Bacteria	p≤0.0001	NSD	NSD	NSD
Any BV	p=0.0041	NSD	NSD	NSD

* No statistical significance determined
**Number of ureaplasma positives too small for meaningful p value.
No statistical differences were seen in the vaginal deliveries

In an earlier study, of 609 women delivering by cesarean section with membranes intact, we saw similar results. *Ureaplasma* species, *M. hominis*, *G. vaginalis*, *Lactobacillus* sp., and *Peptostreptococcus* were isolated consistently more often in the spontaneous group and were more often associated with histologically or clinically diagnosed cases of chorioamnionitis suggesting that these vaginal organisms crossed the choriodecidual space and then crossed the intact chorioamniotic membranes into the amniotic fluid.

Validity of Mycoplasma Methods

In analyzing the data from this study, it was determined that in vaginal cultures collected at the first prenatal and at delivery, our isolation rate of *Mycoplasma hominis* was below what had been previously published. Our isolation rate was 10.4% at the prenatal visit and 5.5% at delivery. Our placental isolation rate was also less than what we had previously described in other populations. We therefore sought to determine if there were problems in the collection, storage, shipping and processing of specimens.

We conducted two separate experiments to compare the detection rate of *M. hominis* by culture with that of PCR to determine if freezing and thawing had adversely affected the integrity of the specimen. 84 vaginal specimens collected in PCR transport buffer (1xPBS pH 7.4) at the time of enrollment were tested by PCR for the presence of *M. hominis*. 9/84 (10.7%) were positive by PCR and this was 100% in agreement with the culture results of specimens collected at the same time. We then collected 81 additional

samples which were tested by culture and PCR but in addition to the PCR buffer sample an aliquot of culture medium (SP4) was also tested by PCR to control for sampling errors from specimens collected with two different swabs. 6/81 specimens were positive by culture and PCR (PCR buffer and media). Three additional specimens were positive by PCR in both samples but were negative by culture. Sampling errors could have contributed to this discrepancy. One additional specimen was PCR positive in the buffer sample and negative in the media sample which might explain the negative culture result. 10/81(12.3%) were positive for *M. hominis* in this experiment. Total agreement between culture and PCR for the two experiments was 159/163 or (97.5%). Culturally, *M. hominis* was detected in 9% of the study population and by PCR the detection rate increased to 11.5%. However, regardless of the method, it appears that in this population the *M. hominis* detection rate was somewhat less than the 40-50% seen in other studies. The isolation rate of ureaplasma in this subset of patients 101/165 (61%) did not seem to be affected. It should be noted that all specimens positive for *M. hominis* by culture and/or PCR were also culturally positive for ureaplasma . PCR and culture results for ureaplasma were 100% in agreement.

New Spcecies Detected

Since this study cultured not only placentas from cesarean sections with intact membranes but cesarean sections with ruptured membranes and randomly selected vaginal deliveries, the microorganisms isolated from the placenta represent a wide array of flora naturally occurring in the female genital tract. During the course of the study, there were 29 aerobic and microaerophilic organisms that could not be identified by routine methods used in our laboratory or at the Wadsworth Anaerobe Laboratory at the UCLA VA Hospital in Los Angeles. These organisms have been sent to MIDI labs for sequencing (16s ribosome analysis) and are considered to be new organisms not previously identified. These organisms were not found in their MicroSequence Database, GenBank or RDP. These unknown organisms were isolated from placentas over a four year period so contamination from a common source is not likely. Five of the 14 strict anaerobes appeared unique on initial analysis. Future research will include examining and elucidating the role these fastidious organisms play in BV, chorioamnionitis and spontaneous abortions.

In addition, there were fifteen species of lactobacillus that were unusual. On initial rib typing analysis, two of these species appear unique and never before isolated or described. Also, there were thirteen Lactobacillus species GB Y16329 isolated from human placentas. These bacteria have never been described in humans before and their significance is unknown. Further analysis of these organisms will be completed and the results of this work will be published in the future.

Pregnancy Outcomes

This study afforded us the opportunity to follow a large cohort of women as to their delivery outcome. In addition to the women that met our study criteria and had their placentas

and cord blood submitted for culture workup, we collected outcome data on the delivery status of all women that enrolled in the UAB study at their prenatal visit. This information was crucial in determining the overall pregnancy outcomes for this population. The outcomes of those deliveries are summarized in Appendix IV.

Active Duty versus Non-Active Duty

One of the major goals of this study was to compare the outcomes of active duty women to those of non-active duty or dependents and to determine if ureaplasma chorioamnion colonization was associated with premature birth in active duty military personnel and whether this explains the observed difference in the rate of prematurity between active duty and dependent women. As mentioned earlier, enlisted women (from the placental population) were 2.4 times (95%CI = 1.3, 4.5) the odds of having a preterm delivery $\leq$ 34 weeks as compared to not enlisted. Officers were 1.4 times (95%CI = 0.3, 6.4) as likely to have a preterm delivery $\leq$ 34 weeks. This holds true after controlling for possible confounding variables. The same holds true when looking at vaginal and placental flora of the delivery group (n=761). A statistical significance was seen when comparing the detection rate of *Ureaplasma* species ($p \leq 0.0001$) detected at prenatal and *Ureaplasma* species detected at delivery (p=0.0008) as well as *Ureaplasma* species from the placental tissue (p=0.0085) of active duty women versus non-active duty women. Although, this is representative of all deliveries and not analyzed by delivery type, these differences should be considered when addressing predictors of adverse outcomes.

Table 5

Vaginal Flora and Placental Isolation of *Ureaplasma* species in Active Duty Women vs. Non-Active Duty Women Within the Placental Population

	Active Duty N=176	**Non-Active Duty N=502**	**p-value**
Ureaplasma prenatal vaginal	128/173 (74%)	275/491 (56 %)	$p \leq 0.0001$
BV prenatal	41/173 (23.7%)	95/494 (19.2%)	NSD
Ureaplasma delivery vaginal	117/164 (71.3%)	272/482 (56.4%)	p=0.0008
BV delivery	36/175 (20.6%)	80/500 (16%)	NSD
Ureaplasma species/placental	36/176 (20.5%)	62/502 (12.4%)	p=0.0085
Neisseria gonorrhoeae	0	0	
Trichomonas vaginalis	6/175 (3.4%)	13/492 (2.6%)	NSD
Chlamydia trachomatis	5/101 (5%)	5/320 (1.6%)	p=0.0512

In a previous study of 609 women who delivered by cesarean section with membranes intact, *Ureaplasma* species was the most common organism isolated from the chorioamnion. Ureaplasma alone or in the presence of other bacteria in chorioamnion was associated with histologic chorioamnionitis. Furthermore, ureaplasmal infection of the chorioamnion in the absence of other bacteria was associated with birth <37 weeks and $\leq$ 34 weeks even after multifactorial analysis to adjust for labor and other obstetric and demographic factors that could confound the association. Presence of organisms in

the chorioamnion in association with chorioamnionitis was inversely related to gestational age and birthweight.

Of the 4,291 women enrolled in our study, there were 343 reported SAB's (9.5%). When univariate and multivariate modeling was completed, no single bacteria or combination of organisms isolated from the vaginal secretions at the first prenatal visit were significantly associated with SAB's. However, when assessing IUFD's, a statistical significance was seen when analyzing women colonized in the cervix with *Ureaplasma* species and also positive for BV. Women having both *Ureaplasma* species and BV were 2.8 times (95%CI=1.1, 7.2) the odds of having a stillbirth than women who had only BV or ureaplasma. This data holds true even after adjusting for all other variables in the model (race/ethnicity, marital status, age, military status, etc.).

BV

BV is a clinical condition caused by replacement of normal hydrogen peroxide producing Lactobacillus sp. in the vagina with high concentrations of characteristic sets of aerobic and anaerobic bacteria. The cause of BV is unknown, but the epidemiology of the syndrome suggests that it is sexually associated. Others have reported that BV has been associated with various complications of pregnancy including spontaneous abortion, preterm labor, premature birth, preterm premature rupture of membranes, amniotic fluid infection, postpartum endometritis, and post cesarean wound infections. (McGregor, Hillier, Donders, Andinkra, Schwebke). In Hillier's study and other's, women with BV were more likely to be unmarried, to be black, to have low incomes and to have previously low-birth-weight infants. Although in this study we report the rate of detection of BV to be 21% in the prenatal group and 17% in the delivery group, we saw no correlation with poor outcomes. In the present study, using univariate and multivariate analysis, we did not find an association of BV detected at prenatal or at delivery associated with adverse pregnancy outcomes including spontaneous abortion, intrauterine fetal demise, preterm labor at $\leq$ 34 weeks or < 37 weeks, low birth weight (<2500 grams or <1000 grams). The reason for the disparate results could be due to the demographics of the study population. Women being seen at NMCSD (4,291) participating in this study were only 13% black, 12% unmarried, 6.9% previous preterm infant and 18% previously having low-birth-weight infants. As for low income, regardless of the pay scale of women or their sponsors, all women had access to prenatal care.

It has also been reported by Hillier and others that the association of *Mycoplasma hominis* and BV are associated with low-birth-weight infants and increased risk of early pregnancy loss. In this study, although the diagnosis of BV was in agreement with other reported studies, the isolation rate of *M. hominis* was much lower overall than what has previously been reported. When looking at just those women that had BV at their prenatal visit, 321/867 (37%) were also colonized with *M. hominis*. Of those women followed through to delivery, 18/101 (17.8%) were diagnosed with BV and colonized with *M. hominis*. Those women having BV and *M. hominis* at their prenatal screening were more likely to have low-birth-weight (<2500 grams) infants ($p \leq 0.0001$) and

preterm (<37 weeks) deliveries (p=0.009). A statistical significance was also seen in low-birth-weight infants born to women with BV and *M. hominis* at delivery (p=0.0029) but this did not hold true for any other infant adverse outcome.

This study was designed to determine if the presence of BV was a risk factor for ureaplasmal invasion of the chorioamnion. Women who had BV either at their prenatal or delivery screening, were not at greater odds for the presence of ureaplasma in their placenta at delivery (OR = 1.2, CI = 0.8-2.0). We also looked at whether organisms commonly associated with BV (*Bacteroides, Prevotella, G. vaginalis, Peptostreptococcus, Lactobacillus* and *M. hominis*) were also more likely to be isolated from the placenta of women diagnosed with BV. We found that women diagnosed with BV at prenatal and/or delivery were more likely to have *G. vaginalis* (p<=0.0001) isolated from the placenta at delivery regardless of delivery route. However, when this organism was isolated from the placenta, it did not appear to be associated with adverse outcomes (p=0.8808) (gestational age <37 weeks, birthweight <2500 gms, IUFD, infant death). Women who tested positive for prevotella in their placenta were 6.4 times more likely to have a preterm birth ≤34 weeks when compared to women who did not test positive. However, due to the small number of cases (nF) the confidence interval was wide (1.2-35.1), and therefore caution should be exercised in interpreting these results.

Cord Blood

In evaluating infants for early onset sepsis, cord blood is cultured for most bacteria but ureaplasma is not routinely sought. As part of this study to evaluate the role of chorioamnion infection in adverse pregnancy outcome in military women and dependent women, cord blood was collected and processed within an hour of delivery. 326 cord bloods were collected in the delivery room (C group) and were analyzed for ureaplasma and other pathogens. 129 of these cord bloods were collected in duplicate in the laboratory (D group) after decontamination of the cord with 70% EtOH. The list of microorganisms detected in each group are found in Appendix VI. In each group, ureaplasma was the most common organism isolated from cord blood 63/326 (19%) in the C group and 15/129 (11.6%) in the D group. In the C group, 32/63 (51%) ureaplasma isolates were found in pure culture and in the D group 12/15 (80%) were found in pure culture. Many of the organisms isolated from these sites could be considered vaginal contaminants. However, finding them in pure culture argues against that fact.

Knowing that bacterial infections have been associated with adverse pregnancy outcomes, we were especially interested in the outcomes of those mothers and infants whose cord blood were culturally positive. Outcomes selected (Table 6) are those generally thought to be associated with bacterial infections. The infants with one or more microorganisms cultured from cord blood were significantly associated with adverse pregnancy outcome (p = 0.032) in the C group when analyzed by chi ^{2} (95%CI). None of the thirty-six bacterial species identified were associated significantly when compared individually to adverse pregnancy outcomes. When analyzing the D group, infants having a cord blood positive for any bacteria showed an increase in preterm birth ≤ 34 weeks (p=0.0143).

Table 6

ADVERSE INFANT OUTCOMES USED IN ANALYSIS

RULE OUT SEPSIS (ROS)
RESPIRATORY DISTRESS SYNDROME (RDS)
TEMPORARY TRANSIENT TACHYPNIA
NEONATAL DEATH
INTRAUTERINE FETAL DEMISE (IUFD)
INFANT PNEUMONIA
SMALL GESTATIONAL AGE (SGA)

When comparing the cultural data from the cord bloods with that of the corresponding placental tissue, ureaplasma was again the most common organism isolated from both sites. 24/63 (38%) ureaplasma isolates from the C group were also found in the corresponding placental culture and 5/15 (33%) of the D group were found in the corresponding placenta. When at least one organism isolated from the cord blood was also isolated from the corresponding placenta and adverse outcomes were assessed, the infants were at increased risk for adverse outcomes ($p=0.022$) in group C. The numbers were to small in the D group for statistical analysis. Only 3/15 patients having cord bloods positive with ureaplasma and positive placentas had adverse outcomes. Two of those patients had suspected sepsis and 1 patient was low birthweight ($\leq$34 weeks) and also had suspected sepsis.

Infant Nasal Colonization

At the time of delivery, vaginal swabs were collected on all participating women and nasal swabs were collected on each infant to further study the carriage rate of ureaplasma and *M. hominis* in the vaginal vault of pregnant women and the incidence of nasal colonization of their infants. Infant outcomes were also assessed. The vaginal isolation rate of ureaplasma and *M. hominis* at delivery were 426/750 (57%) and 37/750 (5%) respectively. The placental isolation rate of ureaplasma was 13.5% and for *M. hominis* was 1.4%. The detection rate of these organisms from the nasal passages of infants was 149/750 (20%) for ureaplasma and 8/750 (1%) for *M. hominis*.

There was a statistical significance ($p<0.0001$) when looking at those women positive vaginally at delivery for ureaplasma and the detection of ureaplasma in the nasal passage of the infant 136/426 (32%). In the placental population, there were 101 ureaplasma placenta positive Mother/infant pairs analyzed. Of those, 65/101 (64%) of the ureaplasma positive placentas had corresponding infants with ureaplasma positive nasal passages. This was statistically significant ($p<0.0001$).

In looking at adverse outcomes (preterm birth < 37 weeks, $\leq$ 34 weeks; low birth weight, <2500 grams, <1000 grams; and IUFD) associated with infants colonized with ureaplasma in their nasal passages, the two outcomes showing statistical significance were IUFD ($p=0.0045$) and very low birth weight < 1000gms ($p=0.0023$). Of the 17

cases of IUFD in the placental population, 8/17 or 47% were colonized with ureaplasma in the nasal passages. There were 22 infants born weighing < 1000 grams and 10/22 (46%) were colonized in the nasal passage with ureaplasma .

Of the women positive for *M. hominis* vaginally at delivery, 7/37 (19%) had infants colonized with *M. hominis* in their nasal passage. There were a total of eight infants positive for *M. hominis* at birth and the one not in agreement was negative vaginally but positive in the placenta. Additionally, there were five other infants positive for *M. hominis* nasally and positive in the placenta for a total of 6/8 (75%). Due to the low isolation rate of *M. hominis*, infection rate and infant outcomes were not analyzed statistically. However, 4/6 infants colonized in the nasal passage with *M. hominis* and the corresponding placenta was also positive, had one or more of the following outcomes:3/6 sepsis, 2/6 RDS, 1/6 tachypnia, 1/6 pneumonia, 3/6 SGA $\leq$ 34 weeks, 3/6 < 37 weeks, 1/6 birthweight < 1000 grams, 3/6 birthwieght < 2500 grams.

WORK TO BE DONE

- All ureaplasma strains isolated from vaginal secretions and infant nasal passages and placental isolates have been stored at –80°C for future serotyping. This information may provide insight as to the invasiveness of particular serovars.
- Sera collected at both prenatal and delivery time points will be tested for antibodies directed towards ureaplasma .
- Associations seen when looking at antibiotic use in pregnancy still need to be addressed. We wish to look specifically at the drugs (macrolides, fluoroquinolones, tetracycline, clindamycin and metranitazole) that would most likely affect the vaginal flora associated with BV and ureaplasmal and *M. hominis* colonization. This is not an easy task. It requires going back to the original data set pulled from CHCS by Mark Turner (MS Access data set), searching for the drugs in question, creating new variables, linking these records back to the original population and placenta data sets, and creating a "yes, no" variable corresponding to "if you had ANY of the 5 from the list". Then, time would be needed to run the univariate analyses for the different populations and outcomes and bacteria types mentioned. After that, if warranted, a multivariate analyses would need to be completed. Response from NHRC: "We can possibly get to it soon, but I cannot give you a firm date"
- One additional factor (previous preterm birth) needs to be added to the univariate and multivariate analyses

REPORTABLE OUTCOMES AND CONCLUSIONS

Cervicovaginal ureaplasmal infection alone is not predictive of preterm birth. Only a subpopulation of women infected in the lower genital tract are at risk for chorioamnion invasion and premature birth. The major goal of this study was to identify microbiologic factors that may predispose to and/or predict chorioamnion invasion and premature birth. Specifically, we sought to determine if the presence of bacterial vaginosis (BV) was a

risk factor for ureaplasmal invasion of the chorioamnion. We also sought to determine if ureaplasma chorioamnion colonization was associated with premature birth in active duty military personnel and whether this explains the observed difference in the rate of prematurity between active duty and dependent women.

- Patients positive for both BV and colonized vaginally with *Ureaplasma* species at their first prenatal visit was predictive of IUFD
- Patients positive for BV without *M. hominis* colonization was not associated with preterm birth or low- birth -weight infants
- Patients positive for BV and colonized vaginally with *M. hominis* at their first prenatal visit was predictive of preterm birth (< 37 weeks) and low-birth-weight infants (<2500 grams)
- Patients positive for BV with or without *M. hominis* colonization was predictive of *Ureaplasma* species in the placenta.
- Enlisted women (from the placental population) were 2.4 times (CI = 1.3, 4.5) the odds of having a preterm delivery $\leq$ 34 weeks as compared to not enlisted. However, ureaplasma chorioamnion infection did not explain the differences.
- Those women whose labor was spontaneous were more likely to have one or more organisms isolated from their placenta. This holds true to earlier studies.
- Organisms isolated from placentas cultured after vaginal deliveries or c-section with ruptured membranes, are not reflective of those involved in the onset of spontaneous labor. This was evidenced by the diverse number of species and the large number of organisms found in those who delivered vaginally with spontaneous labor versus those who delivered by c-section with ruptured membranes and with those delivering by c-section with intact membranes.
- No conclusions could be determined from the c-section intact membrane group due to small numbers of organisms

Personnel Receiving Pay From Research Effort

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Previously Presented Works:

1. Simplifying Detection of Perinatal Pathogens: Comparison of Clinician-Obtained to Self-Obtained Vaginal Specimens. [1]PD Stamper, [2]GC Gray, [3]LB Duffy, [3]GH Cassell
[1]Naval Medical Center San Diego, San Diego, CA, [2] Naval Health Research Center, San Diego, CA, [3]University of Alabama at Birmingham, Birmingham, AL
Presented at the International Conference on Emerging Infectious Diseases, March 1998, Atlanta, Georgia.

Objective: In a large prospective epidemiological study of pregnant women, clinician-obtained vaginal specimens were compared to self-obtained vaginal specimens with cultures of mycoplasmas and *Trichomonas vaginalis* and the assessment of smears for the detection of bacterial vaginosis (BV).
Methods: Women enrolling in a large prospective epidemiological study of pregnant women at the Naval Medical Center, San Diego were asked to perform self-obtained vaginal specimens and permit a clinician to collect vaginal swabs. The subjects, using sterile swabs, followed specific instructions explained to them by a nurse enroller to obtain vaginal wall specimens. One swab from the clinician-obtained specimen and one swab from the self-obtained specimen was used to culture *Trichomonas vaginalis* (InPouch TV, Biomed Diagnostics Inc.). Bacterial vaginosis was determined with another pair of swabs from a Gram stained smear of vaginal secretions using the Nugent method. For the detection of Ureaplasma urealyticum and mycoplasma species, the third swab was used to inoculate 10B broth which was frozen at -70oC and transported to the Diagnostic Mycoplasma Laboratory at the University of Alabama at Birmingham for quantitative culture in 10 B broth and on A8 plates.
Results: Sixty-seven women consented to the dual vaginal swabbing procedures at their initial visit for prenatal care during October 28 through November 15, 1996. Of those, there were 53 matched pairs of swabs obtained for comparison. All 53 pairs of the *Trichomonas vaginalis* cultures were negative (100% agreement). When evaluated for BV, 51 (96% agreement) of the 53 matched Gram stained pairs were of the same grouping (39 negative, 3 intermediate, 9 positive). Twenty-nine (54.7%) of the 53 subject pairs were culture positive for Ureaplasma urealyticum and/or mycoplasma species (98% agreement).
Conclusion: In this population, there was high agreement between the clinician-obtained and self-obtained vaginal swab cultures and Gram stains. Self-obtained vaginal swab collection may reduce the demands upon clinicians and simplify epidemiological studies of pregnant women.

2. Prenatal Prevalence of Bacterial Vaginosis Among Military Beneficiaries
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Presented at the American College of Obstetrics and Gynecologist and the Association of Women's Health Obstetrics and Neonatal Nurses, Armed Forces District Meeting, Kissimmee, FL, October 1998.

Bacterial vaginosis has been associated with preterm labor and other adverse pregnancy outcomes. The presence or absence of bacterial vaginosis (BV) was evaluated in 2,202 pregnant women at Naval Medical Center San Diego (NMCSD) who had enrolled in a prospective epidemiological study of chorioamnion infection from June 1996 to January of 1998. The use of self-obtained vaginal specimens was validated so that during prenatal screening these women were able to perform vaginal self-swabs for the detection of BV. Data collection and subject follow-up continues. Preliminary prenatal screening data reveal that these military beneficiaries have a normal prevalence of BV, 18% using the Nugent Gram stain method.

A total population of 2,202 were enrolled with complete covariate data for 1,888 women, Active duty women were more likely to have BV:

	Active Duty (n=428)	Non-active (n=1,460)	Odds Ration(95%CI)
BV	23%	17%	1.45 (1.11-1.90)

However, after adjusting for marital status and age, there was no appreciable difference between the prevalence of BV among active duty and non-active duty military beneficiaries.

3. *Ureaplasma urealyticum* is the Most Common Organism Isolated from Cord Blood

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Presented at the 99th American Society for Microbiology, 1999 Chicago, IL

In evaluating infants for early onset sepsis, cord blood is cultured for most bacteria, but *Ureaplasma urealyticum* (*Uu*) is not routinely sought. *Uu* is a commensal organism of the lower genital tract of females, but in a subpopulation of individuals, it can invade the upper genital tract. In this subpopulation, it is a significant cause of chorioamnionitis, is associated with preterm birth and neonatal morbidity and mortality. As part of an on-going study to evaluate the role of chorioamnion infection in adverse pregnancy outcome in military women, cord blood was collected and processed within an hour of delivery. To date, 89 cord bloods have been analyzed for *Uu* and other pathogens. 30/89 specimens (34%) were positive for one or more microorganisms. *Uu* was isolated from 17/89 (19%), 13 of 17 (76%) were in pure culture. The next most common single organism isolated was E. coli (4/89 or 4%). Mycoplasma species was isolated from only one infant and this was in conjunction with *Uu*. The other microorganisms isolated were as follows *Lactobacillus sp.* (3), *P. acnes* (2), *Gardnerella vaginalis* (2), *Bacteroides vulgatus* (2), *Bacteroides caccae* (1), *Propionibacterium sp.* (1) *Peptostreptococcus anaerobius* (1), *Streptococcus alpha hemolytic* (1), *Klebsiella pneumoniae* (1) *E. lentum* (1), *Bacillus species* (1). Given the frequency with which *Uu* occurs in cord blood and

the potential risks, there is need to access the clinical significance of our findings. Because cord blood is increasingly being used for autologous transfusions in premature infants and for unrelated donor hematopoietic stem cell transplantation and ureaplasmas and mycoplasmas are not included in the screening process, the need for additional studies is all the more urgent.

4. RISK FACTORS FOR CHORIOAMNION INFECTION AND ADVERSE PREGNANCY OUTCOME AMONG ACTIVE-DUTY MILITARY WOMEN AND DEPENDENT WOMEN

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To be presented September, 2001 "Women In Military Service: Research on Health for Today and Tomorrow", Military and Veteran Women Health Research.

Up to 20% of enlisted Navy women become pregnant each year and nearly 15% of these pregnancies suffer adverse effects. The percentage is in excess of that experienced in most other US female populations. The difference does not appear to be related to environmental exposures. However, bacterial infections of the lower genital tract may in part explain poor pregnancy outcome. The primary objective of the present study was to determine whether *Ureaplasma urealyticum* (*Uu*) chorioamnion infection was associated with premature birth. A secondary objective was to determine if bacterial vaginosis (BV) was a risk factor for Uu invasion of the chorioamnion. All pregnant women (active duty women and dependents of active duty military personnel) seeking prenatal care at the Naval Medical Center San Diego were eligible for participation in this study. From June of 1996 to April of 1999, 4,312 women were enrolled (4,291 analyzed). Vaginal secretions were collected at the first prenatal visit and were analyzed for the presence of Uu and BV. A subset of women (all women delivering by Cesarean section, all women delivering preterm either by Cesarean or vaginally and randomly selected women delivering vaginally N=761) were followed at delivery and placental tissue and amniotic fluid were cultured for the presence of aerobes, anaerobes and Uu. Also collected at delivery were vaginal secretions for the presence of Uu and BV.

60% of the study population were positive for Uu at first prenatal visit with 21% being positive for BV. Adverse outcomes in this group included 12% having spontaneous abortion (SAB), 1% having intrauterine fetal demise (IUFD), 9% delivering preterm (< 37 weeks gestation) and 3% delivering in $\leq$ 34 weeks gestation. *Uu* vaginal colonization at prenatal or at delivery with the presence or absence of BV was not associated with SAB or preterm delivery. However, multivariate analysis of IUFD's revealed that there was a statistical significance in women colonized with *Uu* and positive for BV. Women having both *Uu* and BV had 2.8 times the odds of having a stillbirth than women who had only *Uu* or BV. This remained true even after adjusting for all other variables in the model (race/ethnicity, marital status, age, etc.). However, women who had BV either at prenatal or delivery, were not at greater odds for presence of *Uu* in their placenta at delivery (OR=1.2, CI=0.8-2.0).

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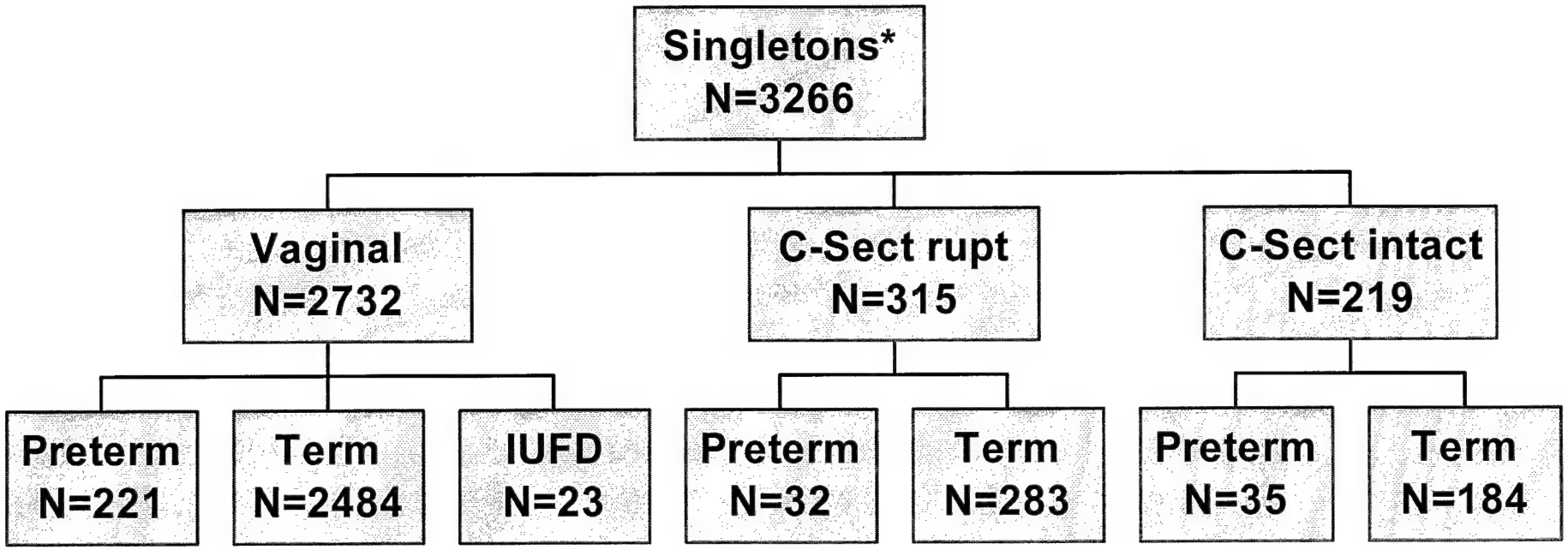
APPENDIX I

Figure 1

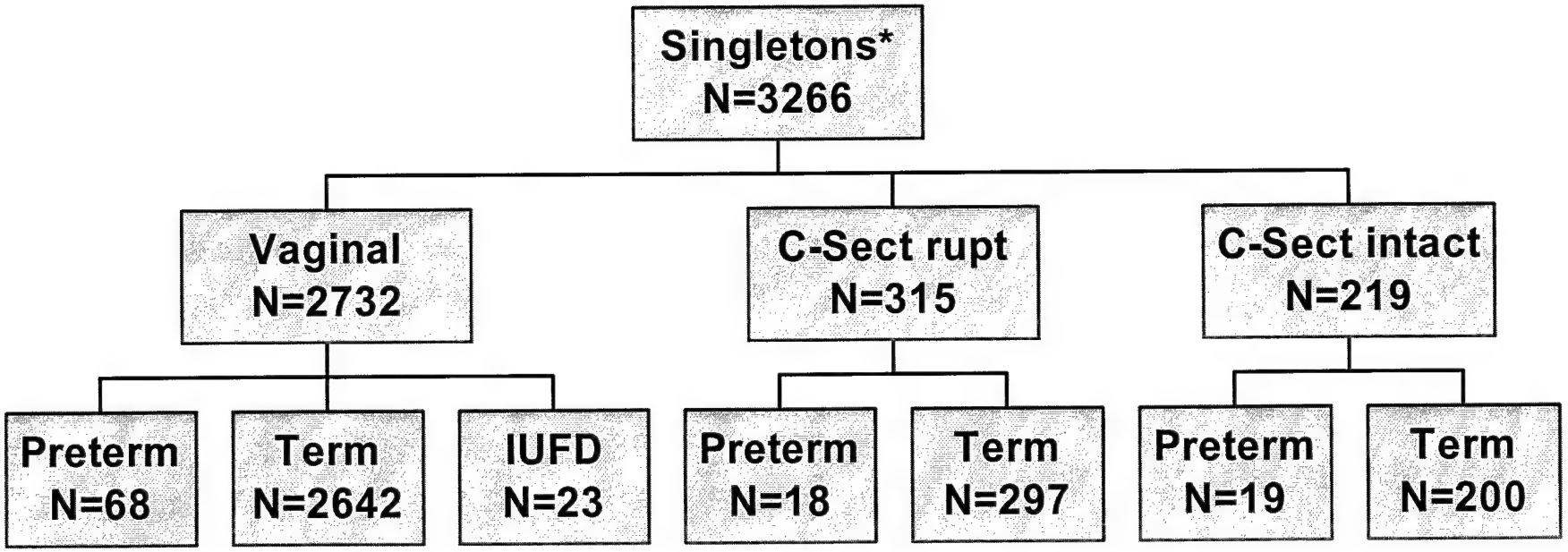
TOTAL DELIVERIES
Preterm Breakdown



Preterm defined as <37 weeks

Figure 2

TOTAL DELIVERIES
Preterm Breakdown



Preterm defined as ≤34 weeks

Figure 3

PRENATAL/DELIVERY
Completed Data Set
(preterm defined as <37 weeks)

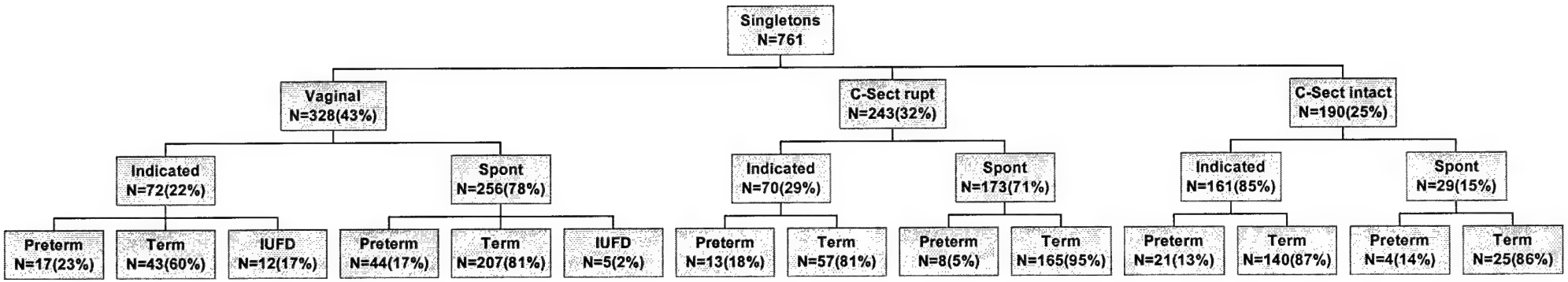
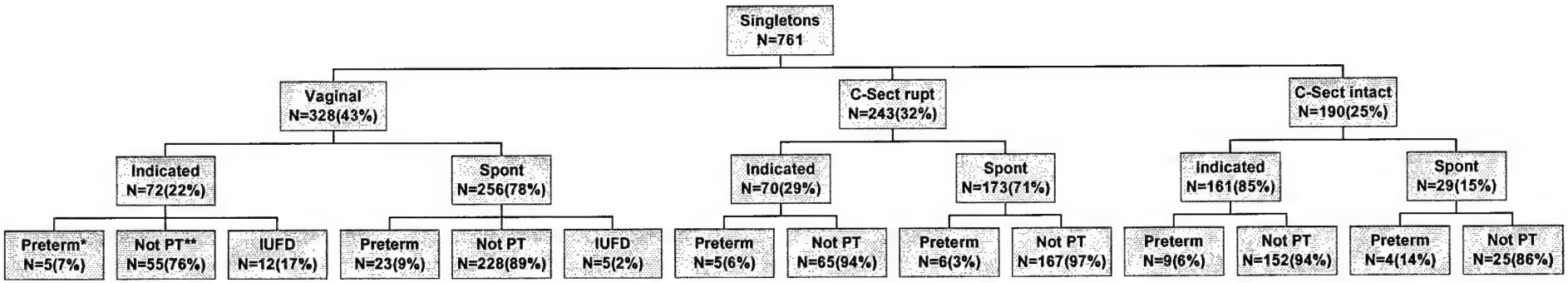


Figure 4

PRENATAL/DELIVERY
Completed Data Set
(preterm defined as ≤34 weeks)



APPENDIX II

Table 1 Univariate modeling chorioamnion study

Independent Variable	SAB (N=4291)* RR† (95% CI)	IUFD (N=3905)* RR (95% CI)	Preterm <37 weeks (N=3874)* RR (95% CI)	Preterm <34 weeks (N=3874)* RR (95% CI)	Low Birthweight <2500 grams (N=3874)* RR (95% CI)	Very Low Birthweight <1000 grams (N=3874)* RR (95% CI)
delivery type						
vaginal			ref	ref	ref	ref
c-section with intact membrane			2.4 (1.6-3.6)	3.7 (2.2-6.3)	3.7 (2.4-5.6)	6.5 (2.0-21.3)
c-section without intact membrane			1.2 (0.8-1.9)	2.1 (1.2-3.5)	1.5 (0.9-2.3)	0.8 (0.1-5.8)
Bv						
No	ref	ref	ref	ref	ref	ref
Yes	1.2 (0.9-1.6)	1.5 (0.6-3.5)	1.0 (0.7-1.4)	1.3 (0.8-2.1)	1.3 (0.9-1.9)	1.5 (0.4-5.8)
presence of bv and uu						
bv or uu	ref	ref	ref	ref	ref	ref
bv and uu	1.2 (0.9-1.7)	3.1 (1.1-8.6)	1.1 (0.8-1.6)	1.3 (0.7-2.2)	1.4 (0.9-2.1)	1.5 (0.3-7.5)
uu and myco						
No	ref	ref	ref	ref	ref	ref
Yes	1.1 (0.7-1.6)	1.8 (0.6-5.2)	1.3 (0.8-1.9)	1.1 (0.5-2.2)	1.6 (1.0-2.6)	0.9 (0.1-6.7)
Uu						
No	ref	ref	ref	ref	ref	ref
Yes	1.1 (0.9-1.5)	1.1 (0.5-2.4)	1.3 (1.0-1.7)	1.4 (0.9-2.2)	1.9 (1.3-2.7)	1.0 (0.3-3.2)
Myco						
No	ref	ref	ref	ref	ref	ref
Yes	2.8 (1.1-6.9)	x	2.1 (0.6-7.2)	x	x	x
Tvag						
No	ref	ref	ref	ref	ref	ref
Yes	2.1 (0.8-5.6)	x	1.0 (0.2-4.4)	1.4 (0.2-10.8)	2.7 (0.8-9.1)	x
Paygrade						
not enlisted	ref	ref	ref	ref	ref	ref
Enlisted	1.1 (0.8-1.4)	1.0 (0.4-2.5)	1.0 (0.7-1.3)	1.3 (0.8-2.0)	1.1 (0.8-1.6)	1.1 (0.3-3.9)
Officer	0.7 (0.3-1.5)	2.7 (0.6-11.5)	1.0 (0.5-2.1)	0.7 (0.2-2.8)	0.4 (0.1-1.5)	2.7 (0.3-21.0)
race/ethnicity						
White	ref	ref	ref	ref	ref	ref
Black	1.1 (0.8-1.5)	2.0 (0.8-4.9)	1.3 (0.9-1.9)	1.7 (1.0-2.8)	1.9 (1.3-2.9)	1.3 (0.3-5.8)
Asian/PI	1.0 (0.7-1.4)	0.5 (0.1-2.3)	1.0 (0.7-1.5)	1.1 (0.6-2.0)	1.0 (0.6-1.6)	2.9 (0.9-9.4)
Hispanic	0.7 (0.5-1.0)	2.2 (0.9-5.2)	1.1 (0.8-1.6)	1.0 (0.6-1.7	0.8 (0.5-1.3)	1.1 (0.2-5.1)
Other	1.1 (0.6-1.7)	2.2 (0.7-7.4)	1.2 (0.7-2.0)	1.3 (0.6-3.0)	1.3 (0.7-2.5)	x
maternal age						
14-19	ref	ref	ref	ref	ref	ref
20-34	0.7 (0.5-0.8)	1.1(0.4-2.9)	0.7 (0.5-1.0)	0.7 (0.5-1.2)	0.6 (0.4-0.8)	0.8 (0.2-3.0)
35+	2.3 (1.6-3.1)	0.9 (0.2-3.9)	1.7 (1.1-2.5)	1.3 (0.7-2.6)	1.8 (1.2-2.9)	0.9 (0.1-7.2)
marital status						
not married	ref	ref	ref	ref	ref	ref
Married	1.0 (0.8-1.4)	0.7 (0.3-1.7)	1.0 (0.7-1.4)	0.7 (0.4-1.2)	1.1 (0.7-1.7)	0.6 (0.2-2.3)
Education						
high school or less	ref	ref	ref	ref	ref	ref
some college	1.2 (1.0-1.5)	1.0 (0.5-2.2)	1.1 (0.8-1.4)	1.4 (0.9-2.1)	1.0 (0.8-1.4)	0.8 (0.3-2.4)
smoking history						
No	ref	ref	ref	ref	ref	ref
Yes	1.1 (0.8-1.5)	1.2 (0.5-3.0)	1.1 (0.8-1.5)	1.2 (0.8-2.0)	1.3 (0.9-1.9)	0.4 (0.1-2.8)
prenatal antibiotic use						
No	ref	ref	ref	ref	ref	ref
Yes	0.9 (0.7-1.2)	2.5 (1.2-5.5)	1.4 (1.1-1.9)	1.8 (1.1-2.9)	1.7 (1.2-2.3)	3.1 (0.6-14.8)

** N;=total for model; SAB=All women, IUFD=All women excluding those with SAB, Preterm and low birthweight=All live births; Actual numbers used in modeling were lower due to missing values.*

† RR = unadjusted relative risk; CI = 95% confidence interval .

"x" indicates cell size inadequate to calculate RR

Table 2 Multivariate modeling chorioamnion study

Independent Variable	SAB (N= 3761)* (sab=343) OR† (95% CI)	IUFD (N= 3513)* (iufd=30) OR (95% CI)	Preterm <37 weeks (N= 2444)* (<37=217) OR (95% CI)	Preterm ≤34 weeks (N= 2415)* (≤34=84) OR (95% CI)	Low Birthweight <2500gms (N= 2387)* (lbw=156) OR (95% CI)	Very Low Birthweight ≤1000gms (N= 1586)* (vlbw=7) OR (95% CI)
bv						
no	x	x	x	x	x	x
yes	x	x	x	x	x	x
presence of bv and uu						
bv or uu	x	ref	x	x	x	x
bv and uu	x	2.8 (1.1-7.2)	x	x	x	x
uu and myco						
no	x	x	x	x	x	x
yes	x	x	x	x	x	x
uu						
no	x	x	x	x	x	x
yes	x	x	x	x	x	x
myco						
no	x	x	x	x	x	x
yes	x	x	x	x	x	x
tvag						
no	x	x	x	x	x	x
yes	x	x	x	x	x	x
paygrade						
not enlisted	x	x	x	x	x	x
enlisted	x	x	x	x	x	x
officer	x	x	x	x	x	x
race/ethnicity						
white	x	ref	x	x	ref	x
black	x	6.7 (1.7-26.5)	x	x	2.4 (1.6-3.7)	x
Asian/PI	x	3.7 (0.7-18.5)	x	x	1.3 (0.8-2.1)	x
Hispanic	x	2.5 (0.4-15.3)	x	x	0.9 (0.5-1.5)	x
other	x	8.2 (1.6-41.1)	x	x	2.0 (1.0-3.6)	x
maternal age						
14-19	ref	x	x	x	x	x
20-34	ref	x	x	x	x	x
35+	2.5 (1.8-3.4)	x	x	x	x	x
marital status						
not married	x	x	x	x	x	x
married	x	x	x	x	x	x
education						
High School or less	x	x	x	x	x	x
Some college	x	x	x	x	x	x
smoking history						
no	x	x	x	x	x	x
yes	x	x	x	x	x	x
prenatal antibiotic use						
no	x	ref	ref	ref	ref	x
yes	x	2.1 (1.0-4.4)	1.7 (1.3-2.3)	1.9 (1.2-3.0)	1.9 (1.3-2.7)	x

** N = total for model; SAB=All women, IUFD=All women excluding those with SAB, Preterm and low birthweight=All live births; Actual numbers used in modeling are lower than population due to missing values.*

† OR = Odds Ratios adjusted for all other covariates in the model; CI = 95% confidence interval .

x = Variables not included in final modeling due to insignificance.

Table 3 Univariate modeling chorioamnion study (placental population)

Independent Variable	Preterm <37 weeks (N=766)* RR (95% CI)	Preterm <34 weeks (N=766)* RR (95% CI)	Low Birthweight <2500 grams (N=766)* RR (95% CI)	Very Low Birthweight <1000 grams (N=766)* RR (95% CI)
delivery type				
vaginal	ref	ref	ref	ref
c-section with intact membrane	1.04 (0.6-1.7)	1.2 (0.6-2.4)	1.4 (0.8-2.3)	1.5 (0.3-8.3)
c-section without intact membrane	0.5 (0.3-0.8)	0.6 (0.3-1.2)	0.5 (0.3-1.0)	x
bv				
no	ref	ref	ref	ref
yes	1.2 (0.7-2.0)	1.3 (0.7-2.7)	1.1 (0.6-2.0)	x
presence of bv and uu				
bv or uu	ref	ref	ref	ref
bv and uu	1.6 (0.9-2.8)	1.2 (0.5-3.3)	1.3 (0.7-2.5)	x
uumyco				
no	ref	ref	ref	ref
yes	1.6 (0.8-3.2)	1.7 (0.7-4.3)	1.9 (0.9-3.9)	x
uu				
no	ref	ref	ref	ref
yes	1.7 (1.1-2.7)	2.2 (1.1-4.4)	1.9 (1.1-3.3)	0.5 (0.1-2.8)
myco				
no	ref	ref	ref	ref
yes	3.0 (0.5-16.8)	x	x	x
tvag				
no	ref	ref	ref	ref
yes	1.0 (0.2-4.5)	1.0 (0.1-8.2)	2.5 (0.7-2.2)	x
paygrade				
not enlisted	ref	ref	ref	ref
enlisted	1.5 (0.9-2.4)	2.4 91.3-4.5)	1.3 (0.8-2.3)	1.6 (0.3-9.0)
officer	1.4 (0.5-3.7)	1.1 (0.2-4.6)	0.6 (0.1-2.7)	4.8 (0.5-42.2)
race/ethnicity				
white	ref	ref	ref	ref
black	1.0 (0.5-1.9)	1.2 (0.5-2.8)	1.3 (0.7-2.6)	x
Asian/PI	0.6 (0.3-1.2)	0.7 (0.3-1.8)	0.8 (0.4-1.7)	1.2 (0.1-10.1)
Hispanic	1.6 (0.9-2.8)	1.4 (0.7-3.1)	0.9 (0.4-1.8)	1.2 (0.1-10.0)
other	0.8 (0.2-2.7)	0.5 (0.1-4.1)	2.4 (0.9-6.1)	x
maternal age				
14-19	ref	ref	ref	ref
20-34	0.9 (0.5-1.5)	0.9 (0.4-1.8)	0.7 (0.4-1.3)	1.4 (0.2-12.2)
35+	1.3 (0.7-2.3)	1.4 (0.6-3.1)	1.6 (0.9-3.0)	1.3 (0.1-11.1)
marital status				
not married	ref	ref	ref	ref
married	0.9 (0.5-1.7)	0.7 (0.3-1.5)	.8 (0.4-1.6)	0.7 (0.1-6.2)
education				
high school or less	ref	ref	ref	ref
some college	1.1 (0.7-1.8)	1.5 (0.8-2.9)	1.1 (0.7-1.8)	1.3 (0.2-7.0)
smoking history				
no	ref	ref	ref	ref
yes	1.1 (0.6-1.9)	1.2 (0.6-2.5)	1.8 (1.0-3.1)	0.8 (0.1-7.3)
prenatal antibiotic use				
no	ref	ref	ref	ref
yes	2.3 (1.3-4.1)	1.8 (0.9-3.7)	1.8 (1.0-3.2)	3.3 (0.4-29.6)

** N;=total for model; Preterm and low birthweight=All live births; Actual numbers used in modeling were lower due to missing values.*

† RR = unadjusted relative risk; CI = 95% confidence interval .

"x" indicates cell size inadequate to calculate RR

Table 4 Multivariate modeling chorioamnion study (placental population)

Independent Variable	Preterm <37 weeks (N= 590)* (<37=14) OR (95% CI)	Preterm ≤34 weeks (N= 508)* (≤34=38) OR (95% CI)	Low Birthweight <2500gms (N= 582)* (lbw=67) OR (95% CI)	Very Low Birthweight ≤1000gms (N= 575)* (vlbw=7) OR (95% CI)
bv				
no	x	x	x	x
yes	x	x	x	x
presence of bv and uu				
bv or uu	x	x	x	x
bv and uu	x	x	x	x
uumyco				
no	x	x	x	x
yes	x	x	x	x
uu				
no	ref	x	ref	x
yes	1.7 (1.0-2.9)	x	1.8 (1.1-3.0)	x
myco				
no	x	x	x	x
yes	x	x	x	x
tvag				
no	x	x	x	x
yes	x	x	x	x
paygrade				
not enlisted	x	ref	x	x
enlisted	x	2.4 (1.3-4.5)	x	x
officer	x	1.4 (0.3-6.4)	x	x
race/ethnicity				
white	x	x	x	x
black	x	x	x	x
Asian/PI	x	x	x	x
Hispanic	x	x	x	x
other	x	x	x	x
maternal age				
14-19	x	x	x	x
20-34	x	x	x	x
35+	x	x	x	x
marital status				
not married	x	x	x	x
married	x	x	x	x
education				
High School or less	x	x	x	x
Some college	x	x	x	x
smoking history				
no	x	x	x	x
yes	x	x	x	x
prenatal antibiotic use				
no	ref	x	x	x
yes	2.2 (1.3-3.7)	x	x	x

** N = total for model; Preterm and low birthweight=All live births; Actual numbers used in modeling are lower than population due to missing values.*

† OR = Odds Ratios adjusted for all other covariates in the model; CI = 95% confidence interval .

x = *Variables not included in final modeling due to insignificance.*

Table 6 Multivariate modeling chorioamnion study (placental population)

Independent Variable	Preterm <37 weeks (N= 601)* (<37=83) OR (95% CI)	Preterm ≤34 weeks (N= 659)* (≤34=46) OR (95% CI)	Low Birthweight <2500gms (N= 749)* (lbw=82) OR (95% CI)	Very Low Birthweight ≤1000gms (N= 744)* (vlbw=8) OR (95% CI)
bv				
no	x	x	x	x
yes	x	x	x	x
presence of bv and uu				
bv or uu	x	x	x	x
bv and uu	x	x	x	x
uumyco				
no	x	x	x	x
yes	x	x	x	x
uu				
no	x	x	x	x
yes	x	x	x	x
myco				
no	ref	x	x	x
yes	10.2 (1.6-64.4)	x	x	61.0 (9.3-398.9)
paygrade				
not enlisted	x	ref	x	x
enlisted	x	2.4 (1.3-4.5)	x	x
officer	x	1.4 (0.3-6.4)	x	x
race/ethnicity				
white	x	x	x	x
black	x	x	x	x
Asian/PI	x	x	x	x
Hispanic	x	x	x	x
other	x	x	x	x
maternal age				
14-19	x	x	x	x
20-34	x	x	x	x
35+	x	x	x	x
marital status				
not married	x	x	x	x
married	x	x	x	x
education				
High School or less	x	x	x	x
Some college	x	x	x	x
smoking history				
no	x	x	ref	x
yes	x	x	1.8 (1.0-3.0)	x
prenatal antibiotic use				
no	ref	x	x	x
yes	2.3 (1.4-3.9)	x	x	x

** N = total for model; Preterm and low birthweight=All live births; Actual numbers used in modeling are lower than population due to missing values.*

† OR = Odds Ratios adjusted for all other covariates in the model; CI = 95% confidence interval .

x = Variables not included in final modeling due to insignificance.

bacteria measured at delivery

APPENDIX III

MICROORGANISMS ISOLATED FROM THE PLACENTA

MICROORGANISM	VAGINAL SPONTANEOUS N=256	VAGINAL INDICATED N=72	CESAREAN RUPTURED SPONTANEOUS N=173	CESAREAN RUPTURED INDICATED N=70	CESAREAN INTACT SPONTANEOUS N=29	CESAREAN INTACT INDICATED N=161
Ureaplasma urealyticum	46 (18.0%)	9 (12.5%)	38 (22.0%)	8 (11.4%)	1 (3.4%)	1 (0.6%)
Gardnerella vaginalis	23 (9.0%)	8 (11.1%)	11 (6.4%)	5 (7.1%)	1 (3.4%)	2 (1.2%)
Lactobacillus species, H2O2+	23 (9.0%)	5 (6.9%)	2 (1.2%)	5 (7.1%)	1 (3.4%)	0
Staphylococcus species, coagulase-neg, not S. saprophyticus	18 (7.0%)	7 (9.7%)	2 (1.2%)	2 (2.9%)	1 (3.4%)	4(2.5%)
Streptococcus, beta-hemolytic Group B	16 (6.3%)	5 (6.9%)	5 (2.9%)	3 (4.3%)	0	0
Streptococcus, Group D non-enterococcus	20 (7.8%)	2 (2.8%)	3 (1.7%)	1 (1.4%)	1 (3.4%)	0
Streptococcus species, alpha-hemolytic	7 (2.7%)	6 (8.3%)	6 (3.5%)	2 (2.9%)	0	2 (1.2%)
Propionibacterium acnes	4 (1.6%)	1 (1.4%)	5 (2.9%)	3 (4.3%)	1 (3.4%)	9 (5.6%)
Corynebacteria species	10 (3.9%)	2 (2.8%)	1 (0.6%)	2 (2.9%)	1 (3.4%)	1 (0.6%)
Lactobacillus species, H2O2-	8 (3.1%)	2 (2.8%)	3 (1.7%)	0	0	2 (1.2%)
Escherichia coli	9 (3.5%)	3 (4.2%)	3 (1.7%)	0	0	0
Peptostreptococcus asaccharolyticus	6 (2.3%)	5 (6.9%)	0	3 (4.3%)	0	0
Mycoplasma species	7 (2.7%)	2 (2.8%)	1 (0.6%)	1 (1.4%)	0	0
Enterococcus, Group D	3 (1.2%)	4 (5.6%)	3 (1.7%)	1 (1.4%)	0	0
Bifidobacterium species	9 (3.5%)	3 (4.2%)	0	0	0	0
Anaerobic microorganism unable to be identified by Wadsworth, MIDI, Genbank, or RDP	4 (1.6%)	3 (4.2%)	1 (0.6%)	1 (1.4%)	0	0
Prevotella bivia (Bacteroides bivius)	7 (2.7%)	1 (1.4%)	1 (0.6%)	0	0	0
Eubacterium aerofaciens	5 (2.0%)	1 (1.4%)	5 (2.0%)	0	0	0
Peptostreptococcus magnus	4 (1.6%)	2 (2.8%)	1 (0.6%)	0	0	1 (0.6%)
Lactobacillus species, 2nd colony type, H2O2+	3 (1.2%)	3 (4.2%)	0	1 (1.4%)	1 (3.4%)	0
Eubacterium lentum	5 (2.0%)	2 (2.8%)	0	1 (1.4%)	0	0
Actinomyces species	7 (2.7%)	0	0	0	0	0
Bacteroides uniformis/ovatus	5 (2.0%)	1 (1.4%)	0	0	0	0
Bacteroides vulgatus	2 (0.8%)	3 (4.2%)	1 (0.6%)	0	0	0
Corynebacteria species, 2nd colony type	5 (2.0%)	1 (1.4%)	0	0	0	0
Peptostreptococcus tetradius	2 (0.8%)	1 (1.4%)	1 (0.6%)	1 (1.4%)	0	0
Micrococcus species	2 (0.8%)	1 (1.4%)	1 (0.6%)	0	0	1 (0.6%)
Streptococcus species, gamma-hemolytic	5 (2.0%)	0	0	0	0	0
Lactobacillus species, GB Y16329, Genbank, H2O2-	3 (1.2%)	1 (1.4%)	1 (0.6%)	0	0	0
Bacteroides thetaiotaomicron	2 (0.8%)	3 (4.2%)	0	0	0	0
Clostridium clostridioforme	2 (0.8%)	3 (4.2%)	0	0	0	0
Mobiluncus species	4 (1.6%)	1 (1.4%)	0	0	0	0
Gardnerella vaginalis, 2nd colony type	2 (0.8%)	2 (2.8%)	0	0	0	0

MICROORGANISM	VAGINAL SPONTANEOUS N=256	VAGINAL INDICATED N=72	CESAREAN RUPTURED SPONTANEOUS N=173	CESAREAN RUPTURED INDICATED N=70	CESAREAN INTACT SPONTANEOUS N=29	CESAREAN NTACT INDICATED N=161
Propionibacterium species not P. acnes	4 (1.6%)	0	0	0	0	0
Streptococcus species, alpha-hemolytic, 2nd colony type	0	2 (2.8%)	1 (0.6%)	0	0	0
Streptococcus species, nutritionally variant	0	0	3 (1.7%)	0	0	0
Klebsiella pneumoniae	1 (0.4%)	1 (1.4%)	1 (0.6%)	0	0	0
Candida albicans	2 (0.8%)	1 (1.4%)	0	0	0	0
Bacteroides species	3 (1.2%)	0	0	0	0	0
Clostridium species	3 (1.2%)	0	0	0	0	0
Peptostreptococcus species	1 (0.4%)	1 (1.4%)	1 (0.6%)	0	0	0
Propionibacterium species	1 (0.4%)	1 (1.4%)	1 (0.6%)	0	0	0
Staphylococcus species, coagulase-neg, not S. saprophyticus 2nd colony type	1 (0.4%)	0	0	1 (1.4%)	0	0
Streptococcus, beta-hemolytic Group G	0	0	1 (0.6%)	1 (1.4%)	0	0
Lactobacillus species, H2O2 unable to determine, non-viable	2 (0.8%)	0	0	0	0	0
Lactobacillus species, H2O2 not determined, MIDI	1 (0.4%)	0	0	0	0	1 (0.6%)
Lactobacillus vaginalis, MIDI, H2O2+	0	2 (2.8%)	0	0	0	0
Bacillus species not B. anthracis or B. cereus	0	1 (1.4%)	1 (0.6%)	0	0	0
Enterobacter cloacae	0	2 (2.8%)	0	0	0	0
Citrobacter freundii	2 (0.8%)	0	0	0	0	0
Actinomyces meyeri	0	2 (2.8%)	0	0	0	0
Actinomyces neuii	1 (0.4%)	1 (1.4%)	0	0	0	0
Bacteroides fragilis	2 (0.8%)	0	0	0	0	0
Bacteroides ovatus	2 (0.8%)	0	0	0	0	0
Prevotella oralis/veroralis (Bacteroides oralis/veroralis)	2 (0.8%)	0	0	0	0	0
Prevotella denticola (Bacteroides denticola)	2 (0.8%)	0	0	0	0	0
Bifidobacterium species, 2nd colony type	2 (0.8%)	0	0	0	0	0
Bifidobacterium adolescentis	1 (0.4%)	0	1 (0.6%)	0	0	0
Clostridium subterminale	2 (0.8%)	0	0	0	0	0
Eubacterium species	2 (0.8%)	0	0	0	0	0
Eubacterium monoforme	1 (0.4%)	1 (1.4%)	0	0	0	0
Peptostreptococcus anaerobius	2 (0.8%)	0	0	0	0	0
Peptostreptococcus asaccharolyticus, 2nd colony type	1 (0.4%)	1 (1.4%)	0	0	0	0
Propionibacterium avidum	0	0	1 (0.6%)	1 (1.4%)	0	0
Propionibacterium granulosum	2 (0.8%)	0	0	0	0	0

MICROORGANISM	VAGINAL SPONTANEOUS N=256	VAGINAL INDICATED N=72	CESAREAN RUPTURED SPONTANEOUS N=173	CESAREAN RUPTURED INDICATED N=70	CESAREAN INTACT SPONTANEOUS N=29	CESAREAN N INTACT INDICATED N=161
Veillonella species	2 (0.8%)	0	0	0	0	0
Veillonella parvula	2 (0.8%)	0	0	0	0	0
Staphylococcus aureus	1 (0.4%)	0	0	0	0	0
Streptococcus, beta-hemolytic Group F	1 (0.4%)	0	0	0	0	0
Streptococcus, beta-hemolytic not Group A,B,C,D,F,G	1 (0.4%)	0	0	0	0	0
Lactobacillus species, MIDI, H2O2-	1 (0.4%)	0	0	0	0	0
Lactobacillus gasseri, MIDI, H2O2-	1 (0.4%)	0	0	0	0	0
Lactobacillus vaginalis, 2nd colony type	0	1 (1.4%)	0	0	0	0
Corynebacteria xerosis	1 (0.4%)	0	0	0	0	0
Rothia species	0	1 (1.4%)	0	0	0	0
Brevibacterium species	1 (0.4%)	0	0	0	0	0
Corynebacteria psuedogenitalium, GenBank	1 (0.4%)	0	0	0	0	0
Listeria monocytogenes	0	0	0	0	0	0
Escherichia coli 2nd colony type	0	1 (1.4%)	0	0	0	0
Klebsiella oxytoca	1 (0.4%)	0	0	0	0	0
Proteus mirablilis	1 (0.4%)	0	0	0	0	0
Eikenella corrodens	1 (0.4%)	0	0	0	0	0
Capnocytophaga species, DF-1	1 (0.4%)	0	0	0	0	0
Haemophilus influenzae	1 (0.4%)	0	0	0	0	0
Actinomyces odontolyticus	1 (0.4%)	0	0	0	0	0
Actinomyces turicencis	1 (0.4%)	0	0	0	0	0
Actinomyces species, APL 10	1 (0.4%)	0	0	0	0	0
Bacteroides uniformis	1 (0.4%)	0	0	0	0	0
Bacteroides caccae	1 (0.4%)	0	0	0	0	0
Prevotella bivia (Bacteroides bivius), 2nd colony type	1 (0.4%)	0	0	0	0	0
Bacteroides capillosus	1 (0.4%)	0	0	0	0	0
Prevotella melaninogenica (Bacteroides melaninogenica)	1 (0.4%)	0	0	0	0	0
Bacteroides distasonis, 2nd colony type	0	1 (1.4%)	0	0	0	0
Clostridium perfringens	1 (0.4%)	0	0	0	0	0
Clostridium clostridioforme, 2nd colony type	1 (0.4%)	0	0	0	0	0
Clostridium malenominatum	1 (0.4%)	0	0	0	0	0
Clostridium butyricum	1 (0.4%)	0	0	0	0	0
Desulfomonas pigra	1 (0.4%)	0	0	0	0	0
Eubacterium aerofaciens, GenBank	1 (0.4%)	0	0	0	0	0
Fusobacterium species	1 (0.4%)	0	0	0	0	0
Fusobacterium varium	1 (0.4%)	0	0	0	0	0
Ruminococcus gnavus	1 (0.4%)	0	0	0	0	0
Ruminococcus torques	1 (0.4%)	0	0	0	0	0

MICROORGANISM	VAGINAL SPONTANEOUS N=256	VAGINAL INDICATED N=72	CESAREAN RUPTURED SPONTANEOUS N=173	CESAREAN RUPTURED INDICATED N=70	CESAREAN INTACT SPONTANEOUS N=29	CESAREAN INTACT INDICATED N=161
Wolinella species	1 (0.4%)	0	0	0	0	0
Arcanobacterium bernardiae, GenBank	1 (0.4%)	0	0	0	0	0
Stretomyces species	0	1 (1.4%)	0	0	0	0

APPENDIX IV

Table 1 Frequencies for Sample Population

Independent Variable	SAB (N=386)* N (%)†	IUFD (N=31)* N (%)	Preterm (<37wks) (N=293)* N (%)	Preterm (<=34wks) (N=107)* N (%)	Low Birthwt (<2500g) (N=207)* N (%)	Very Low Birthwt (<1000g) (N=15)* N (%)
delivery type						
vaginal			222 (76%)	68 (64%)	147 (71%)	10 (67%)
c-section with intact membrane			35 (12%)	19 (18%)	32 (15%)	4 (27%)
c-section without intact membrane			32 (11%)	18 (17%)	24 (12%)	1 (7%)
missing			4 (1%)	2 (2%)	4 (2%)	0 (0%)
bv						
no	268 (69%)	21 (68%)	230 (78%)	78 (73%)	154 (74%)	8 (53%)
yes	82 (21%)	9 (29%)	55 (19%)	25 (23%)	50 (24%)	5 (33%)
missing	36 (9%)	1 (3%)	8 (3%)	4 (4%)	3 (1%)	2 (13%)
uumyco						
no	349 (90%)	27 (87%)	262 (89%)	96 (90%)	181 (87%)	13 (87%)
yes	37 (10%)	4 (13%)	31 (11%)	11 (10%)	26 (13%)	2 (13%)
uu						
no	173 (45%)	14 (45%)	135 (46%)	47 (44%)	85 (41%)	8 (53%)
yes	176 (45%)	15 (48%)	152 (52%)	57 (53%)	120 (58%)	6 (40%)
missing	37 (10%)	2 (7%)	6 (2%)	3 (3%)	2 (1%)	1 (7%)
myco						
no	343 (89%)	29 (94%)	284 (97%)	104 (97%)	205 (99%)	14 (93%)
yes	6 (2%)	0 (0%)	3 (1%)	0 (0%)	0 (0%)	0 (0%)
missing	37 (9%)	2 (6%)	6 (2%)	3 (3%)	2 (1%)	1 (7%)
tvag						
no	343 (89%)	30 (97%)	283 (97%)	101 (94%)	201 (97%)	13 (87%)
yes	5 (1%)	0 (0)%	3 (1%)	2 (2%)	3 (1%)	0 (0%)
missing	38 (10%)	1 (3%)	7 (2%)	4 (4%)	3 (1%)	2 (13%)
chlamydia						
no	90 (23%)	20 (65%)	173 (59%)	66 (62%)	129 (62%)	7 (47%)
yes	3 (1%)	0 (0%)	6 (2%)	3 (3%)	3 (1%)	1 (7%)
missing	293 (76%)	11 (35%)	114 (39%)	38 (36%)	75 (36%)	7 (47%)
gonorrhea						
no	16 (4%)	5 (16%)	58 (20%)	18 (17%)	44 (21%)	4 (27%)
yes	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
missing	370 (96%)	26 (84%)	235 (80%)	89 (83%)	163 (79%)	11 (73%)
paygrade						
not enlisted	253 (66%)	19 (61%)	199 (68%)	69 (64%)	138 (67%)	9 (60%)
enlisted	81 (21%)	6 (19%)	57 (19%)	25 (23%)	45 (22%)	3 (20%)
officer	7 (2%)	2 (6%)	9 (3%)	3 (3%)	4 (2%)	1 (7%)
missing	45 (12%)	4 (13%)	28 (10%)	10 (9%)	20 (10%)	2 (13%)

Table 1 Frequencies for Sample Population (continued)

Independent Variable	SAB (N=386)* N (%)†	IUFD (N=31)* N (%)	Preterm (<37wks) (N=293)* N (%)	Preterm <=34wks) (N=107)* N (%)	Low Birthwt (<2500g) (N=207)* N (%)	Very Low Birthwt (<1000g) (N=15)* N (%)
race/ethnicity						
white	214 (55%)	9 (29%)	147 (50%)	51 (48%)	95 (46%)	6 (40%)
black	53 (14%)	7 (22%)	44 (15%)	20 (19%)	43 (21%)	2 (13%)
Asian/PI	54 (14%)	3 (10%)	42 (14%)	14 (13%)	29 (14%)	4 (27%)
Hispanic	41 (11%)	7 (22%)	40 (14%)	14 (13%)	21 (10%)	2 (13%)
other	22 (6%)	4 (13%)	18 (6%)	7 (7%)	17 (8%)	1 (7%)
missing	2 (1%)	1 (3%)	2 (1%)	1 (1%)	2 (1%)	0 (0%)
maternal age						
14-19	40 (10%)	3 (10%)	36 (12%)	15 (14%)	34 (16%)	2 (13%)
20-24	123 (32%)	8 (36%)	97 (33%)	34 (32%)	62 (30%)	5 (33%)
25-29	112 (29%)	12 (29%)	78 (27%)	27 (25%)	51 (25%)	6 (40%)
30-34	46 (12%)	5 (16%)	46 (16%)	20 (19%)	34 (16%)	1 (7%)
35+	63 (16%)	2 (6%)	34 (12%)	10 (9%)	24 (12%)	1 (7%)
missing	2 (<1%)	1 (3%)	2 (1%)	1 (1%)	2 (1%)	0 (0%)
marital status						
not married	55 (14%)	6 (19%)	42 (14%)	19 (18%)	30 (14%)	3 (20%)
married	329 (85%)	24 (78%)	249 (85%)	87 (81%)	175 (85%)	12 (80%)
missing	2 (1%)	1 (3%)	2 (1%)	1(1%)	2 (1%)	0 (0%)
education						
high school or less	138 (36%)	11 (36%)	110 (38%)	35 (33%)	83 (40%)	6 (40%)
some college	241 (62%)	19 (61%)	178 (61%)	71 (66%)	121 (58%)	9 (60%)
missing	7 (2%)	1 (3%)	5 (2%)	1 (1%)	3 (1%)	0 (0%)
smoking history						
no	304 (79%)	22 (71%)	233 (80%)	83 (78%)	156 (75%)	14 (93%)
yes	76 (19%)	8 (26%)	56 (19%)	23 (22%)	48 (23%)	1 (7%)
missing	6 (2%)	1 (3%)	4 (1%)	1 (1%)	3 (1%)	0 (0%)
prenatal antibiotic use						
no	142 (37%)	9 (29%)	79 (27%)	28 (26%)	52 (25%)	2 (13%)
yes	149 (39%)	18 (58%)	144 (49%)	59 (55%)	110 (53%)	9 (60%)
missing	95 (25%)	4 (13%)	70 (24%)	20 (19%)	45 (22%)	4 (27%)

** N; overall*

† Due to rounding, some percentages may not add to 100%.

Table 2 Frequencies for Placental Population

Independent Variable	SAB (N=0)* N (%)†	IUFD (N=17)* N (%)	Preterm (<37wks) (N=107)* N (%)	Preterm (<=34wks) (N=52)* N (%)	Low Birthwt (<2500g) (N=83)* N (%)	Very Low Birthwt (<1000g) (N=8)* N (%)
delivery type						
vaginal			65 (57%)	28 (54%)	44 (53%)	6 (75%)
c-section with intact membrane			25 (23%)	13 (25%)	23 (28%)	2 (25%)
c-section without intact membrane			21 (20%)	11 (21%)	16 (19%)	0 (0%)
missing			0 (0%)	0 (0%)	0 (0%)	0 (0%)
bv						
no		13 (76%)	80 (75%)	36 (69%)	61 (73%)	5 (63%)
yes		4 (24%)	25 (23%)	14 (27%)	20 (24%)	2 (25%)
missing		0 (0%)	2 (2%)	2 (4%)	2 (2%)	1 (13%)
uumyco						
no		15 (88%)	93 (87%)	44 (85%)	72 (87%)	7 (88%)
yes		2 (12%)	14 (13%)	8 (15%)	11 (13%)	1 (13%)
uu						
no		7 (41%)	45 (42%)	21 (40%)	34 (41%)	5 (63%)
yes		9 (53%)	60 (56%)	29 (56%)	47 (57%)	2 (25%)
missing		1 (6%)	2 (2%)	2 (4%)	2 (2%)	1 (13%)
myco						
no		16 (94%)	103 (96%)	50 (96%)	81 (98%)	7 (88%)
yes		0 (0%)	2 (2%)	0 (0%)	0 (0%)	0 (0%)
missing		1 (6%)	2 (2%)	2 (4%)	2 (2%)	1 (13%)
tvag						
no		17 (100%)	102 (95%)	48 (92%)	78 (94%)	7 (88%)
yes		0 (0)%	3 (3%)	2 (4%)	3 (4%)	0 (0%)
missing		0 (0%)	2 (2%)	2 (4%)	2 (2%)	1 (13%)
chlamydia						
no		10 (59%)	55 (51%)	30 (58%)	47 (57%)	4 (50%)
yes		0 (0%)	2 (2%)	1 (2%)	0 (0%)	0 (0%)
missing		7 (41%)	50 (47%)	21 (40%)	36 (43%)	4 (50%)
gonorrhea						
no		3 (18%)	27 (25%)	13 (25%)	21 (25%)	2 (25%)
yes		0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
missing		14 (82%)	80 (75%)	39 (75%)	62 (75%)	6 (75%)
paygrade						
not enlisted		12 (71%)	61 (57%)	25 (48%)	51 (61%)	3 (38%)
enlisted		3 (18%)	28 (26%)	19 (37%)	21 (25%)	2 (25%)
officer		0 (0%)	5 (5%)	2 (4%)	2 (2%)	1 (13%)
missing		2 (12%)	13 (12%)	6 (12%)	9 (11%)	2 (25%)

Table 2 Frequencies for Placental Population (continued)

Independent Variable	SAB (N=0)* N (%)†	IUFD (N=17)* N (%)	Preterm (<37wks) (N=107)* N (%)	Preterm (<=34wks) (N=52)* N (%)	Low Birthwt (<2500g) (N=83)* N (%)	Very Low Birthwt (<1000g) (N=8)* N (%)
race/ethnicity						
white		5 (29%)	60 (56%)	29 (56%)	42 (51%)	5 (40%)
black		4 (24%)	14 (13%)	7 (13%)	12 (14%)	0 (0%)
Asian/PI		0 (0%)	10 (9%)	5 (10%)	10 (12%)	1 (13%)
Hispanic		4 (24%)	19 (18%)	9 (17%)	10 (12%)	1 (13%)
other		3 (18%)	4 (4%)	2 (4%)	8 (10%)	1 (13%)
missing		1 (6%)	0 (0%)	0 (0%)	1 (1%)	0 (0%)
maternal age						
14-19		1 (6%)	8 (7%)	4 (8%)	7 (8%)	0 (0%)
20-24		5 (29%)	34 (32%)	16 (31%)	23 (28%)	3 (38%)
25-29		7 (41%)	33 (31%)	13 (25%)	28 (34%)	3 (38%)
30-34		3 (18%)	17 (16%)	11 (21%)	10 (12%)	1 (13%)
35+		0 (0%)	15 (14%)	8 (15%)	14 (17%)	1 (13%)
missing		1 (6%)	0 (0%)	0 (0%)	1 (1%)	0 (0%)
marital status						
not married		2 (12%)	13 (12%)	8 (15%)	11 (13%)	1 (13%)
married		14 (82%)	94 (88%)	44 (85%)	71 (86%)	7 (88%)
missing		1 (6%)	0 (0%)	0 (0%)	1 (1%)	0 (0%)
education						
high school or less		5 (29%)	39 (36%)	17 (33%)	31 (38%)	2 (25%)
some college		11 (65%)	68 (64%)	35 (67%)	51 (61%)	6 (75%)
missing		1 (6%)	0 (0%)	0 (0%)	1 (1%)	0 (0%)
smoking history						
no		12 (71%)	86 (80%)	40 (77%)	59 (71%)	7 (88%)
yes		4 (24%)	21 (20%)	12 (23%)	23 (28%)	1 (13%)
missing		1 (6%)	0 (0%)	0 (0%)	1 (1%)	0 (0%)
prenatal antibiotic use						
no		4 (24%)	23 (22%)	13 (25%)	23 (28%)	1 (13%)
yes		10 (59%)	57 (53%)	31 (60%)	44 (53%)	6 (75%)
missing		3 (18%)	27 (25%)	8 (15%)	16 (19%)	1 (13%)

** N; overall*

† Due to rounding, some percentages may not add to 100%.